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Control of G_1 Arrest After DNA Damage

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The temporal relationship between DNA damage and DNA replication may be critical in determining whether the genetic changes necessary for cellular transformation occur after DNA damage. Recent characterization of the mechanisms responsible for alterations in cell-cycle progression after DNA damage in our laboratory have implicated the p53 (tumor suppressor) protein in the G_1 arrest that occurs after certain types of DNA damage. In particular, we found that levels of p53 protein increased rapidly and transiently after nonlethal doses of γ irradiation (XRT) in hematopoietic cells with wild-type, but not mutant, *p53* genes. These changes in *p53* protein levels were temporally linked to a transient G_1 arrest in these cells. Hematopoietic cells with mutant or absent *p53* genes did not exhibit this G_1 arrest, through they continued to demonstrate a G_2 arrest. We recently extended these observations of a tight correlation between the status of the endogenous *p53* genes and this G_1 arrest after XRT and this cell-cycle alteration after XRT was then established by transfecting cells lacking endogenous *p53* genes with a wild-type gene and observing acquisition of the G_1 arrest and by transfecting cells processing endogenous wild-type *p53* genes with a mutant *p53* gene and observing loss of the G_1 arrest after XRT. These observations and their significance for our understanding of the mechanisms of DNA damage-induced cellular transformation are discussed.

Transient alterations in cell-cycle progression after DNA damage are well documented. Presumably, these responses permit optimal repair of damage before the cell reinitiates replicative DNA synthesis (G_1 arrest) and/or begins mitosis (G_2 arrest). If replicative DNA synthesis or mitosis occurred before repair of the damage, then mutagenic lesions could be "fixed" and propagated (*1*) and could contribute to the progressive increase in genomic changes necessary for neoplastic transformation (Fig. 1). Currently, little is known about the cellular signals required for these cell-cycle check points after DNA damage in mammalian cells. In yeast, the *RAD9* gene product appears to be necessary for G_2 arrest after damage (*2*), but the factors required for G_1 arrest remain unclear.

We recently began to characterize some of the mechanisms that control cell-cycle changes in response to DNA damage in mammalian cells (*3*). Using hematopoietic cell lines as models, we found that nonlethal doses of γ irradiation (XRT) transiently inhibit replicative DNA synthesis via both G_1 and G_2 arrests. We reasoned that this inhibition of replicative DNA synthesis after XRT could result from either inhibition of a positive regulator of DNA synthesis or stimulation or a negative regulator. Because the *p53* gene product had been demonstrated to be a negative regulator of DNA synthesis (*4–7*) and because this tumor-suppressor gene is the most commonly mutated gene thus far identified in human cancers, linkage of *p53* to this DNA damage-induced inhibition of DNA replication was an attractive possibility.

Because the wild-type *p53* gene product is a negative regulator of DNA synthesis, if it were involved in this pathway we would expect to see an increase in p53 protein levels (and/or functional activity) after DNA damage.

Using either a sensitive flow cytometric assay for p53 protein expression or metabolic labeling followed by immunoprecipitation, we observed that levels of p53 increased in ML-1 myeloblastic leukemia cells [which have wild-type *p53* genes (*3*)] in temporal association with the decrease in replicative DNA synthesis after XRT. We demonstrated that this increased p53 protein after XRT was localized to the cell nucleus, which is when *p53* protein must be in order to be functional (*7,8*). Levels of p53 protein also increased in temporal association with a decrease in replicative DNA synthesis after treatment of ML-1 cells with actinomycin D, which, like XRT, induces DNA strand breaks. In contrast, p53 protein levels did not change after exposure of ML-1 cells to cytosine arabinoside, which neither directly damages DNA nor causes a G_1 arrest.

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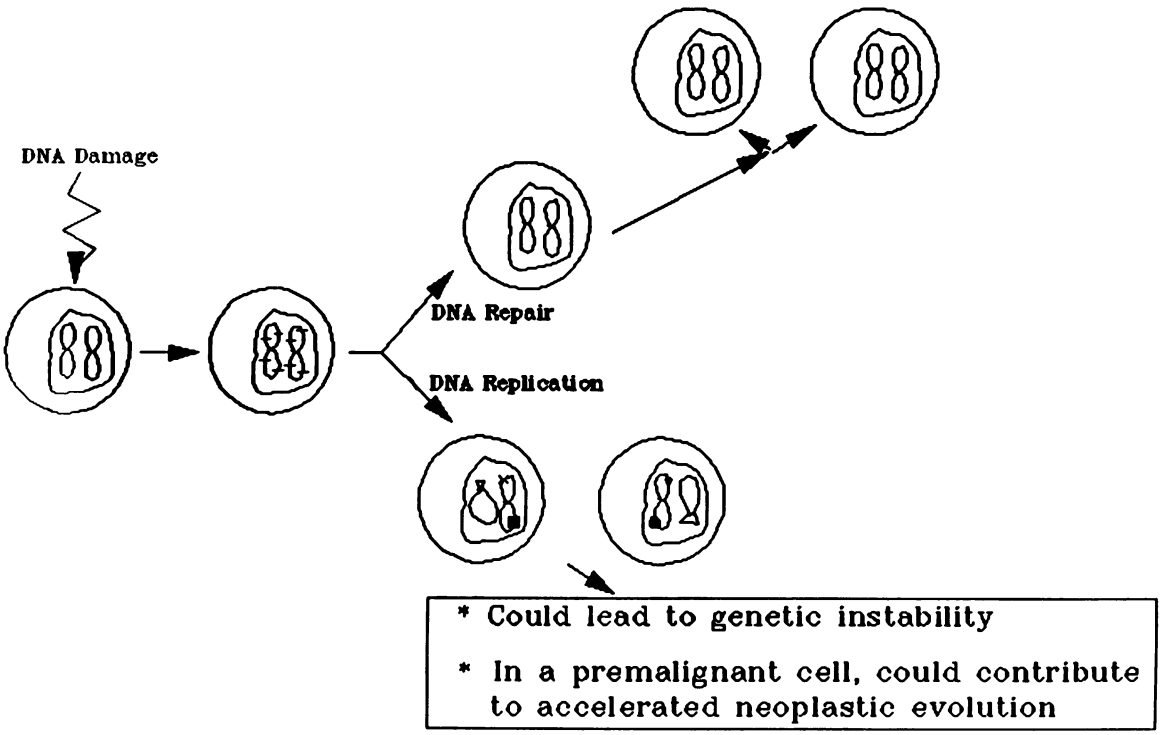

FIGURE 1. Schematic representation of alternative cellular responses to DNA damage. After DNA damage, cells might never replicate again or might die-these two possibilities do not lead to the development of malignancies and are not depicted. The other two possible courses are for the cell to repair the DNA damage before replicative DNA synthesis or for DNA replication to occur before the integrity of the DNA is restored. If the latter option occurs, replicative synthesis will use a damaged DNA template: this could lead to propagation of genetic abnormalities in daughter cells. In a premalignant cell, this latter process could contribute to the array of genetic changes that lead to the fully transformed phenotype. Our data suggest that wild-type p53 protein plays a critical role in determining whether the cell follows the repair or replication pathway.

These results suggested that p53 protein was participating in the cellular response to DNA damage. We then took advantage of the known status of the *p53* gene in a number of hematopoietic cell lines and further implicated wild-type p53 protein in this G1 arrest after XRT by demonstrating that cells with no *p53* genes and cells with mutant *p53* genes do not exhibit the G_1 arrest. Interestingly, however, cells with mutant *p53* genes continued to arrest in G_2 after XRT.

More recently, we demonstrated that this close correlation between *p53* gene status and the ability to arrest in G_1 after XRT is not restricted to hematopoietic cells. Adherent cells with wild-type *p53* genes (normal human fibroblasts, RKO colorectal carcinoma cells, and U-2OS osteosarcomal cells) also exhibit G_1 arrest after XRT, whereas adherent cells with mutant *p53* genes (SW480 colorectal carcinoma cells and Saos osteosarcoma cells) continue to progress though S-phase after XRT (*9*). Thus, with 11 different cell types evaluated thus far, there is a 100% concordance between the status of the *p53* gene and the ability to arrest in G_1 after XRT (summarized in Table 1).

These observations established a very strong correlation, but not a cause-effect relationship, between wild-type *p53* and the G_1 arrest after XRT. Such a cause-effect relationship was established by transfecting *a*) HL60 cells, which have no endogenous *p53* genes, with a wild-type *p53* gene; the transfected cells expressed low levels of p53 protein and partially recovered the G_1 arrest after XRT and *b*) RKO colorectal carcinoma cells, which have wild-type *p53* genes, with a dominant negative mutant *p53* gene; these transfectants partially lost the ability to arrest in G_1 after XRT (*9*). These transfection results confirmed the role of wild-type *p53* genes in the arrest of cells in G_1 after XRT, thus allowing cells with wild-type *p53* genes to avoid replication of DNA using a damaged template.

We are currently investigating the biochemical mechanism(s) responsible for this induction of *p53* protein levels after DNA damage. Because we found that levels of p53 mRNA do not change after XRT and because the p53 protein levels also increase after DNA damage induced by doses of actinomycin D that can significantly inhibit RNA synthesis, this induction of p53 protein appears to occur via a post-transcriptional mechanism. In addition, treatment of irradiated cells with caffeine inhibits both the G_1 and G_2 arrests and also inhibits the increase in p53 protein; in contrast, cycloheximide treatment (reversibly) inhibits G_1, but not G_2, arrest, while also blocking the increase in p53 protein after XRT (*3*). These observations suggest that certain types of DNA damage initiate a signal trans-

Table 1. Status of *p53* gene, protein, and γ-irradiation response in selected cell types.

Cell	Lineage	*p53* gene	Mutant codon	Amino acid change	p53 protein[a]	G_1 arrest[b]
NBMP	Myeloid	WT	-	-	+/-	+
ML-1	Myeloid	WT	-	-	+	+
344	Fibroblast	WT	-	-	+	+
RKO	ColoCa	WT	-	-	+	+
U-20S[6]	Osteosarcoma	WT	-	-	+	+
KG-1a	Myeloid	M/-	225	VAL-ILE	-	-
HL-60	Myeloid	A	-	-	-	-
Raji	Lymphoid	M/WT	213	ARG-GLN	+++	-
RPMI8402	Lymphoid	M/WT	273	ARG-CYS	+++	-
SW480	ColoCa	M/-	273 309	ARG-HIS PRO-SER	+++	-
Saos-2(5)	Osteosarcoma	A	-	-	-	-

Abbreviations: NBMP, normal bone marrow progenitor cells rapidly growing in liquid culture (only myeloid progenitor cells grow under these conditions); M, mutant *p53* gene; WT, wild-type *p53* gene; A, absent *p53* gene.

[a]Relative levels of p53 protein; +++, high levels; +, low levels; -, no detectable protein; +/-, proliferative NBMP cells normally have no detectable p53 protein, but express low levels after γ-irradiation.

[b]Significant decrease in percentage of cells in S-phase after γ-irradiation.

duction pathway that transiently increases levels of p53 protein via a post-transcriptional mechanism, which subsequently results in a transient inhibition of replicative DNA synthesis. The effects of caffeine and cycloheximide on this process further demonstrate that exposure to non-genotoxic agents can affect this signal transduction pathway and alter this cellular response to DNA damage and thus increase the risk of "fixing" mutagenic lesions. Investigations to further characterize this signal transduction pathway are underway.

Before these investigations, the contribution of abnormalities in the *p53* gene to tumorigenesis was primarily attributed to the loss of an inhibitor of cellular proliferation, which thus permitted unrestricted cell growth. This mechanism may, in fact, be very important in cellular transformation; however, our observations, in addition to beginning to clarify the important components of the cell-cycle check points after DNA damage, suggest another mechanism for the contribution of abnormalities in the *p53* gene to tumorigenesis and genetic instability. Cells with abnormal *p53* genes do not appear to be able to efficiently cease replicative DNA synthesis after (at least with certain types of) DNA damage, despite the presence of a damaged DNA template. Such cells would be at increased risk for developing further genetic abnormalities after exposure to DNA-damaging agents. A preneoplastic cell with an abnormal *p53* gene might therefore be at higher risk for developing the subsequent genetic changes necessary for expression of a fully transformed phenotype after DNA damage than a cell with wild-type *p53* genes. Thus, the separate observations that *p53* is mutated in a high percentage of human solid tumors and that most tumors contain multiple, gross chromosomal abnormalities may be more than just coincidence; they may be causally related through the type of mechanism discussed here.

In terms of the theme of this symposium, this type of model suggests that both DNA damage and cell proliferation are important contributors to the carcinogenic process and that the relative timing of the two events may be a critical variable. Interestingly, *p53* is a heavily methylated gene [(*10*); Kuerbitz and Kastan, unpublished results], and a high percentage of *p53* mutations in tumors are C to T transitions (*10*), which could result simply from spontaneous deamination of 5-methyldeoxycytidine in the DNA. Thus, genotoxic damage need not be responsible for the *p53* gene mutations that are frequently noted in tumors, and increased cell proliferation alone could increase the chance of such spontaneous deaminations resulting in C to T transitions. According to our model, once activating mutations have occurred in the *p53* gene, whether by genotoxic damage or by spontaneous deamination and a C to T transition, the cell would be at increased risk for developing the other genetic lesions required for a fully transformed phenotype after exposure to DNA damaging agents.

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